Page 2

DIKAN

31. A method for combinatorial cassette-based recombination, comprising:

conjoining a plurality of recombination sites to a plurality of subsequences of at least one nucleic acid, thereby producing a plurality of recombination cassettes;

recombining the recombination cassettes, or fragments thereof, at the recombination sites, thereby producing a plurality of permutations of the recombination cassettes within a plurality of resulting recombinant nucleic acids; and,

selecting the plurality of recombinant nucleic acids for one or more property or encoded activity.

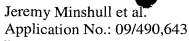
- 32. The method of claim 31, wherein the plurality of recombination cassettes comprise at least one set of functionally similar nucleic acid subsequences.
- 33. A method for permuting subsequences of interest in at least one nucleic acid, the method comprising:

identifying functionally similar subsequences in the at least one nucleic acid; conjoining at least one recombination site to a plurality of the functionally similar subsequences, thereby producing a plurality of recombination cassettes; and,

recombining the recombination cassettes, or fragments thereof, at the recombination sites, thereby producing a plurality of permutations of the recombination cassettes within a plurality of resulting recombinant nucleic acids.

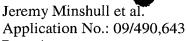
- **34.** The method of claim <u>33</u>, further comprising selecting the plurality of recombinant nucleic acids for one or more property or encoded activity.
- 35. The method of claim 32 or 33, wherein a plurality of different recombination sites are conjoined to a plurality of different types of functionally similar subsequences, wherein each of the different types of subsequences encode functionally different subsequences.
 - **36.** The method of claim 32 or 33, wherein the plurality of recombination sites provide for recombination between functionally similar nucleic acid subsequences, without providing for recombination between functionally dissimilar nucleic acid subsequences.

July 37



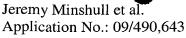
- 37. The method of claim 32 or 33, wherein the plurality of recombination sites provide for recombination between a plurality of different types of functionally similar nucleic acid subsequences.
- **38.** The method of claim 31 or 33, wherein a plurality of different recombination sites are conjoined to a plurality of different nucleic acid subsequences, thereby producing the plurality of recombination cassettes, wherein the plurality of recombination cassettes comprises a plurality of types of cassettes, wherein the types of cassettes each comprise functionally similar sequences.
- **39.** The method of claim 38, wherein the recombining comprises recombining recombination cassettes encoding functionally similar nucleic acid subsequences.
- **40.** The method of claim 39, wherein the recombining comprises recombining a plurality of types of recombination cassettes, each type encoding functionally similar nucleic acid subsequences, wherein the recombination is performed between members of the same type.
- **41.** The method of claim 31 or 33, wherein a plurality of different recombination sites are conjoined to a plurality of different types of nucleic acid subsequences, wherein a plurality of similar recombination sites are conjoined to a plurality of similar nucleic acids.
- **42.** The method of claim 31 or 33, wherein the plurality of recombination sites are provided by incorporating the recombination sites as subsequences of a PCR primer, which PCR primer is extended by a polymerase in a PCR reaction to amplify a portion of the at least one nucleic acid, thereby producing the recombination cassette.
- **43.** The method of claim 42, wherein the recombination sites which are incorporated into the PCR primer are between about 20 and about 40 nucleotides in length.
- 44. The method of claim 31 or 33, wherein the recombination sites are between about 20 and about 40 nucleotides in length.
- **45.** The method of claim 31 or 33, wherein at least one recombination site is located 5' to a coding sequence in the cassette.





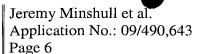
- **46.** The method of claim 31 or 33, wherein at least one recombination site is located 3' to a coding sequence in the cassette.
- 47. The method of claim 31 or 33, wherein at least one recombination site brackets at least one coding sequence in at least one cassette.
- **48.** The method of claim 31 or 33, wherein at least one recombination site is located 5' to at least one coding sequence in at least one cassette and at least one recombination site is located 3' to the at least one coding sequence.
- **49.** The method of claim 31 or 33, wherein the recombination cassettes are produced by hybridizing PCR primers to the at least one nucleic acid, which PCR primers comprise a first region complementary to the at least one nucleic acid and a second region which is not complementary to the at least one nucleic acid.
- **50.** The method of claim 49, wherein the region which is not complementary to the at least one nucleic acid comprises one or more recombination site.
- **51.** The method of claim 31 or 33, wherein the at least one nucleic acid corresponds to a gene cluster.
- **52.** The method of claim 31 or 33, wherein the at least one nucleic acid corresponds to or encodes a multi-subunit enzyme.
- **53.** The method of claim 31 or 33, wherein the plurality of permutations of the recombination cassettes comprises all possible combinations of the recombination cassettes.
- **54.** The method of claim 31 or 33, further comprising recombining one or more of the plurality of recombinant nucleic acids with one or more additional nucleic acid.
- 55. The method of claim 31 or 33, further comprising fragmenting the recombination cassettes with a nuclease prior to said recombining step, wherein said recombining step is performed by primerless PCR.





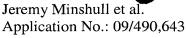
- **56.** The method of claim 31, wherein the recombination cassettes encode functionally related polypeptides, which functionally related polypeptides show low sequence homology.
- 57. The method of claim 31 or 34, further comprising recombining the recombinant nucleic acid with one or more additional nucleic acid and selecting one or more further recombined nucleic acid for one or more additional property or encoded activity.
- **58.** The method of claim 57, further comprising one or more additional cycles of recombination or selection using the further recombined nucleic acid as a substrate for at least one of the additional cycles.
- **59.** The method of claim 57, wherein recombining the recombinant nucleic acid comprises in vitro recombination of the recombinant nucleic acid with one or more additional nucleic acids.
- **60.** The method of claim 59, wherein the in vitro recombination comprises recursive in vitro recombination.
- **61.** The method of claim 57, wherein recombining the recombinant nucleic acid comprises in vivo recombination of the recombinant nucleic acid with one or more additional nucleic acids.
- **62.** The method of claim 60, wherein the in vivo recombination comprises recursive in vivo recombination.
- **63.** The method of claim 31 or 33, wherein the at least one nucleic acid comprises one or more sequence produced by in vitro sequence recombination.
- **64.** The method of claim 31 or 33, wherein the at least one nucleic acid comprises one or more sequences produced by recursive in vitro recombination.
- **65.** The method of claim 31 or 33, wherein the at least one acid is produced by in vivo recombination.
- **66.** The method of claim 31 or 33, wherein the at least one nucleic acid is produced by recursive in vivo sequence recombination.





- 67. The method of claim 31 or 33, wherein the at least one nucleic acid is produced by mutation.
- 68. The method of claim 67, wherein the first nucleic acid is produced by error prone PCR.
- **69.** The method of claim 3<u>1</u> or 33, wherein the cassettes are recombined by fragmentation, hybridization of the fragments to each other or to a template nucleic acid, and elongation of the resulting hybridized fragments.
- **70.** The method of claim <u>69</u>, wherein the fragments are denatured prior to hybridization to each other or to the template nucleic acid.
- 71. The method of claim 69, wherein the fragments are denatured, prior to hybridization, by heating the fragments to between about 80°C and 100°C.
- 72. The method of claim 69, wherein the fragments are hybridized by incubating the fragments, or the fragments and the template, at between about 20°C and about 75°C.
- **73.** The method of claim 69, wherein the fragments are hybridized in the presence of PEG or a salt.
- 74. The method of claim 69, wherein the fragments are hybridized in the presence PEG at a concentration between about 0% and 20% PEG.
- 75. The method of claim 69, wherein the fragments are hybridized in the presence PEG at a concentration between about 5% and 10% PEG.
- **76.** The method of claim 31 or 33, wherein the cassette is cloned into a vector which supplies one or more of: a promoter, a polyadenylation sequence, or a regulatory sequence.
- 77. The method of claim 31 or 33, wherein the plurality of recombination cassettes comprise subsequences which are allelic or species variants.
- 78. The method of claim 31 or 33, wherein the at least one nucleic acid is derived from a cell which is selected for one or more of: pathogenicity, substrate range, environmental hardiness,





presence of one or more key intermediates, ease of genetic manipulation, or likelihood of promiscuous transfer of genetic information to other organisms.

- **79.** The method of claim 31 or 33, wherein the at least one nucleic acid comprises one or more of: a plasmid, a cosmid, a chromosome, an episome, a YAC, a phage, a filamentous phage, a phage P1 clone, or a viral vector.
- **80.** The method of claim 31 or 33, wherein the one or more nucleic acid comprises genomic DNA.
- **81.** The method of claim 31 or 33, wherein the plurality of recombination cassettes comprise amplified genomic DNA.
- **82.** The method of claim 31 or 33, wherein the at least one nucleic acid comprises a metabolic pathway nucleic acid which encodes at least one metabolic pathway.
- 83. The method of claim 31-or 33, wherein the at least one nucleic acid encodes a multi-subunit enzyme.
- **84.** The method of claim 31 or 33, wherein the plurality of recombination cassettes comprise variants of a single gene.
- **85.** The method of claim 31 or 33, wherein the plurality of recombination cassettes comprise variants of more than one gene.
- **86.** The method of claim 31 or 33, wherein the at least one nucleic acid is selected from one or more libraries of nucleic acids derived from one or more of: a bacteria, an *Alcaligenes*, a *Zoogloea*, a *Rhizobium*, a *Bacillus*, an *Azobacter*, or a eukaryote.
- 87. The method of claim 31 or 33 wherein the at least one nucleic acid is derived from one or more cell which comprises a biphenyl catabolizing pathway.
- **88.** The method of claim 31 or 33 wherein the at least one nucleic acid encodes one or more dioxygenase enzyme.



- 89. The method of claim 31 or 33 wherein the at least one nucleic acid encodes one or more biphenyl dioxygenase enzyme.
- **90.** The method of claim 31 or 33 wherein the at least one nucleic acid encodes one or more toluene dioxygenase enzyme.
- 91. The method of claim 31 or 33 wherein the at least one nucleic acid encodes one or more of: a keto reductase, an acyl carrier protein, or a keto synthase.
- **92.** The method of claim 31 or 33, wherein the at least one nucleic acid encodes a regulatory gene.
- 93. The method of claim 31 or 33 wherein the at least one nucleic acid encodes one or more enzyme selected from the group consisting of: an acylase, a dioxygenase, a monooxygenase, a carotenoid synthetic enzyme, a hydrolytic enzyme, a catabolic enzyme, a dibenzothiopene catabólizing enzyme, a nitroreductase, a benzene degrading enzyme, a nitrobenzene degrading enzyme, a nitrotoluene degrading enzyme, a toxin degrading enzyme, an industrial chemical degrading enzyme, an herbicide degrading enzyme, a cellulose degrading enzyme, a pesticide degrading enzyme, a pollutant degrading enzyme, a xylene degrading enzyme, a toluene degrading enzyme, a camphor degrading enzyme, a naphthalene degrading enzyme, a halogenated hydrocarbon degrading enzyme, a biphenyl degrading enzyme, a polychlorinated biphenyl (PCB) degrading enzyme, a polycyclic aromatic hydrocarbon (PHA) degrading enzyme, a polyhydróxybutyrate (PHB) degrading enzyme, a trichlorethylene degrading enzyme, a pentachlorophenyl (PCP) degrading enzyme, a trichloroethylene degrading enzyme, a paranitrobenzyl, esterase, a sesquiterpene synthase, an expandase, a penicillin amidase, a penicillin G amidase, an enzyme which modifies 7-aminodeacetooxycephalosporanic acid (7-ADZA), an enzyme which modifies a semi-synthetically produced cephalosporin, and an enzyme which modifies penicillin V.
- **94.** The method of claim 93, wherein the enzyme is a polyhydroxybutyrate (PHB) degrading enzyme, wherein the first nucleic acid or the template is derived from one or more of: an

Sult Ce

Page 9

Alcaligenes bacteria, a Zoogloea bacteria, a Rhizobium bacteria, a Bacillus bacteria, or an Azobacter bacteria.

- **95.** The method of claim 93, wherein the enzyme is a biphenyl degrading enzyme and wherein the enzyme is expressed in at least one host cell which comprises a biphenyl catabolizing pathway.
- **96.** The method of claim 93, wherein the enzyme is a cellulose degrading enzyme and wherein the first nucleic acid or the template is derived from one or more *Agrobacterium tumefaciens*.
- 97. The method of claim 93, wherein the enzyme is a carotenoid synthetic enzyme and wherein the first nucleic acid or the template is derived from one or more of: Myxococcus xanthus, Rhodobacter sphaeroides, Thermus thermophilus, Erwina uredovora, Haematococcus pluvialis, E. coli, E. herbicola, and R. capsulatus.
- **98.** The method of claim 31 or 33, wherein the further recombined selected nucleic acid encodes one or more enzyme which is resistant to inactivation by one or more epoxide.
- **99.** The method of claim <u>31 or 33</u>, wherein at least one of the recombinant nucleic acids provides one or more organism, when expressed in the organism, with a new or improved ability to convert a pollutant into a nutrient source.
- 100. The method of claim 31 or 33, wherein at least one of the recombinant nucleic acids provides one or more organism, when expressed in the organism, with a new or improved ability to degrade one or more toxin, industrial chemical, herbicide, pesticide or pollutant.
- 101. The method of claim 100, wherein the one or more toxin, industrial chemical, herbicide or pollutant comprises one or more of: benzene, xylene, toluene, camphor, naphthalene, a halogenated hydrocarbon, a polychlorinated biphenyl (PCB), a polycyclic aromatic hydrocarbon (PHA), a trichlorethylene, a pentachlorophenyl (PCP) or trichloroethylene.

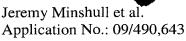


- The method of claim 31 or 33, wherein at least one of the recombinant nucleic acids encodes an enzyme with an improved catalytic activity, a new catalytic activity, altered substrate recognition, thermostability, stability in a non-aqueous solvent, or an altered expression level.
- 103. The method of claim 31 or 33, wherein at least one of the recombinant nucleic acids provides one or more organism, when expressed in the organism, with a new or improved resistance to the presence of one or more heavy metal.
- 104. The method of claim 31 or 33, wherein at least one of the recombinant nucleic acids provides one or more organism, when expressed in the organism, one or more property selected from the group consisting of: modified growth rate, ability to secrete a desired compound, an ability to tolerate an increased temperature, and an ability to tolerate one or more environmental stress.
- 105. The method of claim 31 or 33, wherein at least one of the recombinant nucleic acids provides one or more organism, when expressed in the organism, with a new or improved ability to desulfurize oil.
- 106. The method of claim 31 or 33, wherein at least one of the recombinant nucleic acids provides one or more organism, when expressed in the organism, with a new or improved ability to reduce an organo-nitro compound or to permit the organism to survive in the presence of an organo-nitro compound.
- 107. The method of claim 31 or 33, wherein at least one of the recombinant nucleic acids provides one or more organism, when expressed in the organism, with new or improved utilization of a nutrient source.
- 108. The method of claim 107, wherein the nutrient source is selected from the group consisting of: lactose, whey, galactose, mannitol, xylan, cellobiose, cellulose and sucrose.

109. The method of claim 107, wherein the improved utilization of a nutrient source provides for production of compounds selected from the group consisting of: ethanol, tryptophan, a rhamnolipid surfactant, xanthan gum, polysaccharide xanthan gum and polyhydroxylalkanoate.

- 110. The method of claim 31 or 33, wherein at least one of the recombinant nucleic acids provides one or more organism, when expressed in the organism, new or improved production of one or more product selected from the group consisting of: ethanol, tryptophan, a rhamnolipid surfactant, xanthan gum, polysaccharide xanthan gum, polyhydroxylalkanoate, phenylalanine, and 2-keto-L-gluconic acid.
- 111. The method of claim 31 or 33, wherein at least one of the recombinant nucleic acids provides one or more organism, when expressed in the organism, with a new or improved ability to produce one or more metabolic intermediate.
- 112. The method of claim 111, wherein the metabolic intermediate is selected from the group consisting of: an antibiotic, a vitamin, an amino acid, phenylalanine, an aromatic amino acid, ethanol, butanol, polysaccharide xanthan gum, xanthan gum, bacterial cellulose, a peptide, and a lipid.
- 113. The method of claim 31 or 33, wherein at least one of the recombinant nucleic acids encodes an enzyme which produces one or more compound selected from the group consisting of: a polyketide, a dye, a vitamin, an antibiotic, a carotenoid, a terpenoid, and an isoprenoid.
- 114. The method of claim 113, wherein the dye is indigo.
- 115. The method of claim 113, wherein the vitamin is vitamin C.
- 116. The method of claim 113, wherein the antibiotic is selected from the group consisting of: a peptide, a peptidolactone, a thiopeptide, a beta-lactam, a glycopeptide, a lantibiotic, a microcin, a polyketide-derived antibiotic, an anthracyclin, a tetracyclin, a





macrolide, an avermectin, a polyether, an ansamycins, chloramphenicol, an aminoglycoside, an aminocyclitol, a polyoxin, an agrocin, mederrhodin, dihydrogranatirhodin, 6-deoxyerythromycin A, isovalerylspiramycin, a hybrid macrolide and an isoprenoid.

- 117. The method of claim 113, wherein the polyketide is an antibiotic.
- 118. The method of claim 113, wherein the polyketide is selected from the group consisting of: tetracycline, erythromycin, an anti-cancer agent, daunomycin, an immunosuppressant, FK506, rapamycin, monesin and avermectin.
- 119. The method of claim 113, wherein the isoprenoid is selected from the group consisting of: an antibacterial isoprenoid and an antifungal isoprenoid.
- The method of claim 113, wherein the carotinoid is selected from the group consisting of: a ketocarotenoid, a myxobacton, a spheroidene, a spheroidenone, a lutein, an astaxanthin, a violaxanthin, a 4-ketorulene, a myxoxanthrophyll, an echinenone, a lycopene, a zeaxanthin, a monoglucoside, a diglucoside, an alpha carotene, a beta carotene, a gamma carotene, a delta carotene, a cryptoxanthin monoglucoside, and a neoxanthin.
- 121. The method of claim 31 or 34, wherein the selecting comprises monitoring bioremediation or biodegredation of one or more toxin, industrial chemical, herbicide, pesticide or pollutant by one or more enzyme encoded by the recombinant nucleic acid or the further recombined selected nucleic acid.
- 122. The method of claim 121, the one or more toxin, industrial chemical, herbicide or pollutant comprising one or more of: benzene, xylene, toluene, camphor, naphthalene, a halogenated hydrocarbon, a polychlorinated biphenyl (PCB), a polycyclic aromatic hydrocarbon (PHA), a trichlorethylene, a pentachlorophenyl (PCP) or trichloroethylene.
- 123. The method of claim $3\underline{1}$ or 34, wherein the selecting comprises monitoring one or more reporter gene selected from the group consisting of: luciferase, green fluorescence protein, and β -galactosidase.



- 124. The method of claim 31 or 34, wherein the selecting comprises monitoring one or more of: fluorescence, bioluminescence, colony size, cell growth rate, a chromogenic substrate, and halo formation.
- The method of claim 31 or 34, wherein the selecting comprises performing an ELISA assay.
- 126. The method of claim 31 or 34, wherein the selecting comprises performing a cell-cell activity assay.
- 127. The method of claim 31 or 34, wherein the selecting comprises monitoring differential expression of a protein or nucleic acid expressed in a screened cell comprising the recombinant nucleic acid or the further recombined selected nucleic acid.
- 128. The method of claim 31 or 34, wherein the selecting comprises performing FACS.
- 129. The method of claim 31 or 34, wherein the selecting comprises performing two-color FACS.
- The method of claim 31 or 34, wherein the selecting comprises monitoring gel microdroplets.
- 131. The method of claim 31 or 34, wherein the selecting comprises detecting one or more molecule by mass spectometry.
- 132. The method of claim 31 or 34, wherein a cell comprising the recombinant nucleic acid, or the further recombined selected nucleic acid, is selected in a chemostat.
- 133. The method of claim 31 or 34, wherein the selecting comprises selecting for one or more of: an improved catalytic activity, a new catalytic activity, altered substrate recognition, thermostability, stability in a non-aqueous solvent, or an altered expression level.



- 134. The method of claim 31 or 34, wherein the selecting comprises selecting one or more organism comprising the recombinant nucleic acid for one or more property selected from the group consisting of: a modified growth rate, an ability to secrete a desired compound, an ability to tolerate an increased temperature, and an ability to tolerate one or more environmental stresses.
- 135. The method of claim 31 or 34, wherein the selecting comprises monitoring the presence or absence of one or more secondary metabolite selected from the group consisting of: a polyketide, a dye, a vitamin, an antibiotic, a carotenoid, a terpenoid, and an isoprenoid.
- 136. The method of claim 135, wherein the dye is indigo.
- 137. The method of claim 135, wherein the vitamin is vitamin C.
- The method of claim 135, wherein the antibiotic is selected from the group consisting of: a peptide, a peptidolactone, a thiopeptide, a beta-lactam, a glycopeptide, a lantibiotic, a microcin, a polyketide-derived antibiotic, an anthracyclin, a tetracyclin, a macrolide, an avermectin, a polyether, an ansamycins, chloramphenicol, an aminoglycoside, an aminocyclitol, a polyoxin, an agrocin, mederrhodin, dihydrogranatirhodin, 6-deoxyerythromycin A, isovalerylspiramycin, a hybrid macrolide and an isoprenoid.
- The method of claim 135, wherein the polyketide is an antibiotic.
- 140. The method of claim 135, wherein the polyketide is selected from the group consisting of: tetracycline, erythromycin, an anti-cancer agent, daunomycin, an immunosuppressant, FK506, rapamycin, monesin and avermectin.
- 141. The method of claim 135, wherein the isoprenoid is selected from the group consisting of: an antibacterial isoprenoid and an antifungal isoprenoid.
- 142. The method of claim 135, wherein the carotinoid is selected from the group consisting of: a ketocarotenoid, a myxobacton, a spheroidene, a spheroidenone, a lutein, an astaxanthin, a violaxanthin, a 4-ketorulene, a myxoxanthrophyll, an echinenone, a lycopene, a



zeaxanthin, a monoglucoside, a diglucoside, an alpha carotene, a beta carotene, a gamma carotene, a delta carotene, a cryptoxanthin monoglucoside and a neoxanthin.

Sub Et

143. The method of claim 31 or 34, wherein the selecting comprises monitoring one or more enzymatic activities of one or more enzymes selected from the group consisting of: an acylase, a dioxygenase, a monooxygenase, a carotenoid synthetic enzyme, a hydrolytic enzyme, a catabolic enzyme, a dibenzothiopene catabolizing enzyme, a nitroreductase, a benzene degrading enzyme, a nitrobenzene degrading enzyme, a nitrotoluene degrading enzyme, a toxin degrading enzyme, an industrial chemical degrading enzyme, an herbicide degrading enzyme, a cellulose degrading enzyme, a pesticide degrading enzyme a pollutant degrading enzyme, a xylene degrading enzyme, a toluene degrading enzyme, a camphor degrading enzyme, a naphthalene degrading enzyme, a halogenated hydrocarbon degrading enzyme, a polychlorinated biphenyl (PCB) degrading enzyme, a polycyclic aromatic hydrocarbon (PHA) degrading enzyme, a polyhydroxybutyrate (PHB) degrading enzyme, a trichlorethylene degrading enzyme, a pentachlorophenyl (PCP) degrading enzyme, a trichloroethylene degrading enzyme, a paranitrobenzyl, esterase, a sesquiterpene synthase, an expandase, a penicillin amidase, a penicillin G amidase, an enzyme which modifies 7-aminodeacetooxycephalosporanic acid (7-ADZA), an enzyme which modifies a semi-synthetically produced cephalosporin, and an enzyme which modifies penicillin V.



- 144. The method of claim 31 or 34, wherein the selecting comprises monitoring degradation of one or more of: a toxin, an industrial chemical, an herbicide, a pesticide, a pollutant, PHB, and cellulose.
- 145. The method of claim 144 wherein the one or more toxin, industrial chemical, herbicide or pollutant comprises one or more of: benzene, xylene, toluene, camphor, naphthalene, a halogenated hydrocarbon, a polychlorinated biphenyl (PCB), a polycyclic aromatic hydrocarbon (PHA), a trichlorethylene, a pentachlorophenyl (PCP) or trichloroethylene.
- 146. The method of claim 31 or 34, wherein the selecting comprises monitoring synthesis of one or more carotenoid.

Page 16

- 147. The method of claim 31 or 34, wherein the selecting comprises monitoring resistance of an enzyme to an epoxide.
- 148. The method of claim 31 or 34, wherein the selecting comprises monitoring resistance of a cell modified with the recombinant nucleic acid to a heavy metal.
- 149. The method of claim 31 or 34, wherein the selecting comprises selecting an organism which expresses the recombinant nucleic acid for an ability to desulfurize oil.
- 150. The method of claim 31 or 34, wherein the selecting comprises selecting an organism which expresses the recombinant nucleic acid for an ability to survive in the presence of an organo-nitro compound.
- The method of claim 31 or 34, wherein the selecting comprises selecting an organism for an ability to metabolize lactose, whey, galactose, mannitol, xylan, cellobiose, cellulose or sucrose.
- 152. The method of claim 31 or 34, wherein the selecting comprises selecting an organism for an ability to produce ethanol, tryptophan, a rhamnolipid surfactant, xanthan gum, polysaccharide xanthan gum, polyhydroxylalkanoate, phenylalanine, or 2-keto-L-gluconic acid.

NO NEW MATTER

The above amendments are fully supported by the specification and claims as originally filed and introduce no new matter. For example, support for cassette-based recombination as claimed in claims 31 and 33 is found, e.g., at page 11, line 5-page 13, line 15. Additional support for the various dependent claims is found throughout the specification and claims.

REMARKS

With this amendment, claims 1-30 are canceled and claims 31-152 are pending. It is believed that the new claims are free of the cited prior art as noted in more detail below. To the extent that the rejections are applied to the new claims, Applicants traverse. For

